

SHORT COMMUNICATION

BACTERIAL LEAF SPOT OF CHRIST'S THORN, A NEW DISEASE CAUSED BY *ACIDOVORAX AVENAE* SUBSP. *CITRULLI* IN IRAN

B. Harighi

Plant Protection Department, College of Agriculture and Natural resources, University of Kurdistan, Sanandaj, Iran

SUMMARY

During spring and summer 2004 and 2005, a new disease of Christ's thorn (*Paliurus spina-christi* P. mill.) was observed in Iran, in some areas of the Kurdistan province. Symptoms were initially yellow areas on the leaves, in which irregular water-soaked spots 3-5 mm in diameter developed. A non-fluorescent bacterium was consistently isolated from lesions on King's medium B. Fifteen bacterial isolates were obtained from different locations of west Iran. According to phenotypic, biochemical and physiological properties all bacterial isolates were identified as *Acidovorax avenae* subsp. *citrulli*. They produced round white colonies 1-2 mm in diameter, were all aerobic, gram-, levan-, arginine dihydrolase-, catalase- and potato soft rot-negative, but oxidase-positive, and induced hypersensitivity reaction in tobacco leaves. Pathogenicity of selected isolates was confirmed by injecting bacterial suspension into the underside of leaves. This is the first report of a bacterial disease on Christ's thorn in Iran.

Key words: *Paliurus spina-christi*, bacterial leaf spot, *Acidovorax*, diagnosis.

Christ's thorn (*Paliurus spina-christi* P. Mill.) is a deciduous shrub growing in different locations of the Kurdistan province of Iran. Since 2004, a previously unreported disease was observed in some areas of this province. Symptoms were yellowed area on leaves, within which water-soaked, irregular spots developed. In most cases spots were 3-5 mm in diameter. When examined with a microscope, the cut edge of symptomatic leaves consistently exhibited bacterial streaming. The purpose of this study was to identify and characterize the causal agent of this disease.

Bacterial strains were isolated from infected tissues collected from three locations in eastern Kurdistan in 2004-2005 from May to August. Small pieces bearing

lesions were excised from infected leaves, surface sterilized in 0.25% aqueous sodium hypochlorite for 30 sec, and rinsed in sterile distilled water. Each piece was macerated in 3 ml sterile distilled water for 30 min and resulting suspensions were streaked onto King's medium B (KB) or nutrient agar (NA). Plates were incubated at 28°C and after 3-4 days single colonies were subcultured. Bacterial strains were stored in 15% glycerol at -70°C or cultured on KB and placed at 4°C for short term storage.

Non-fluorescent, gram-negative bacteria were isolated from infected tissues. Strains were levan-negative, oxidase-positive, arginine dihydrolase-negative and induced hypersensitivity reaction on tobacco (*Nicotiana tabacum*). Other biochemical and physiological tests were performed using methods described by Schaad (1988).

Acid production from carbon sources or utilization of amino acids was tested as described by Dye (1968). The physiological and biochemical properties found are summarized in Table 1. The LOPAT (including levan, oxidase, potato soft rot, arginine dihydrolase and tobacco hypersensitivity reaction) tests showed that all isolates belong to *Acidovorax avenae*. According to other phenotypic, biochemical and physiological properties the causal bacterium was identified as *Acidovorax avenae* subsp. *citrulli*, formerly named *Pseudomonas pseudoalcaligenes*.

The species *P. pseudoalcaligenes* was divided into three subspecies namely *P. p.* subsp. *pseudoalcaligenes*, *P. p.* subsp. *citrulli* (inducing water soaked lesion on watermelon) and *P. p.* subsp. *konjaci* (the causal agent of bacterial blight of konjac). The results of numerical analysis and DNA-DNA reassociation experiments (Hu *et al.*, 1991) reclassified subsp. *citrulli* and subsp. *konjaci* as members of a single species, *P. avenae*. Studies by Willems *et al.* (1992) showed that *P. avenae* and its subspecies were to be transferred to the genus *Acidovorax* as *A. avenae* subsp. *citrulli* and *A. konjaci*.

Pathogenicity of four strains was determined by injecting a bacterial suspension into three young *P. spina-christi* plants. Strains were grown in nutrient broth (NB) at 28°C for 48 h. Cells were centrifuged (5 min at 7000 rpm) and pellets were resuspended in sterile-distilled water to a concentration of approximately 1×10^7 CFU ml⁻¹

Table 1. Characterization and identification of the Iranian bacterial isolates in comparison with *A. avenae* subsp. *Citrulli*.

Tests	Bacterial isolates	<i>A. avenae</i> sub. <i>citrulli</i> *
Levan production	-	-
Oxidase activity	+	+
Potato soft rot		-
Arginine dihydrolase	-	-
Tobacco hypersensitivity	+	+
Catalase	-	-
Fluorescent pigment on King B medium	-	-
Alkaline reaction on milk	-	-
Acid reaction on milk	-	-
Ketolactose	-	
Reducing substances from sucrose	-	
Nitrate reduction	+	+
Urease production	+	+
Oxidative/fermentative (O/F)	O	O
Growth at 4°C	-	-
Indole	-	-
H ₂ S from cysteine	+	
H ₂ S from peptone	+	
1% NaCl tolerance	+	
2% NaCl tolerance	-	
Phosphatase	+	
Acetone production	-	
Methyl red	-	
Gas from glucose		-
Hydrolysis of:		
Gelatin		- -
Starch		- -
Casein	-	-
Lecithin	+	-
Tween 80	+	+
Aesculin	-	
Utilization of:		
D-Glucose	+	+
Raffinose	-	-
Sucrose	-	d
Fructose	-	
Ribose	+	+
Erythritol	-	-
D-galactose	+	+
Mannose	+	+
Glycerol	+	
Maltose	-	-
Lactose	-	-
Cellobiose	-	-
Arabinose	+	+
Trehalose	-	-
L-sorbose	-	
Rhamnose	-	-
D-xylose	+	+
Melibiose	+	+
Starch	-	d
Adonitol	-	-

Mannitol	-	-
Dulcitol	-	-
Sorbitol	-	-
Inositol	-	-
Ethanol	-	-
Propionate	-	+
Benzoate	-	-
Malonate	-	-
L-lactate	+	+
D-tartrate	+	+
L-tartrate	+	d
Acetate	-	-
Borate	-	-
Aspartate	+	-
L-arginine	-	-
L-asparagine	+	-
Ornithine	-	-
L-tyrosine	d	d
L-lysine	+	-
L-tryptophan	+	d
Salicin	-	-
Inulin	-	-

^a data selected from Brenner *et al.* (2005), Hu *et al.* (1991) and Willems *et al.* (1992)

+ = 85% or more positive

- = less than 15% positive

d = 15-85% positive

as determined by OD reading ($A_{600} = 0.05$). Plants were incubated in the greenhouse at 25-30°C and 90-98% relative humidity. Bacterial strains were injected into leaf tissue from the lower side using a sterile syringe. Plants were maintained in the greenhouse until symptoms were assessed 14-28 days after inoculation. Plants inoculated with sterile-distilled water only served as control.

All strains tested for pathogenicity produced similar leaf spots on Christ's thorn. The symptoms were observed 2-4 weeks after inoculation. Bacterial strains were re-isolated from infected tissues and biochemical properties determined were similar to those of the original inoculated culture of *A. avenae* subsp. *citrulli*.

Many epidemiological factors of the disease on *P. spina-christi* are still unknown. The disease is favoured by rainy and windy weather. Optimum growth of the bacterium occurs at 25-35°C and warm, dry conditions slow disease development.

REFERENCES

- Brenner D.J., Krieg N.R., Staley J.T., 2005. *Bergey's Manual of Systematic Bacteriology*, 2nd edition. Springer, New York, NY, USA.
- Dye D.W., 1968. A taxonomic study of the genus *Erwinia* 1. The amylovora group. *New Zealand Journal of Science* **11**: 590-607.
- Hu F.P., Young J.M., Triggs C.M., 1991. Numerical analysis and determinative tests for nonfluorescent plant-pathogenic *Pseudomonas* spp., a genomic analysis and reclassification of species related to *Pseudomonas avenae* Manns 1909. *International Journal of Systematic Bacteriology* **41**: 516-525.
- Schaad N.W., 1988. *Laboratory Guide for Identification of Plant Pathogenic Bacteria*. 2nd edition. APS Press, St. Paul, MN, USA.
- Willems A., Goor M., Thielemans S., Gillis M., Kersters K., De Ley J., 1992. Transfer of several phytopathogenic *Pseudomonas* species to *Acidovorax* as *Acidovorax avenae* subsp. *avenae* subsp. nov., comb. Nov., *Acidovorax avenae* subsp. *citrulli*, *Acidovorax avenae* subsp. *cattaleya*, and *Acidovorax konjaci*. *International Journal of Systematic Bacteriology* **42**: 107-119.

